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Quantitative assessment of the efficacy of spiral-wound membrane cleaning procedures to remove biofilms

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ABSTRACT

Cleaning of high pressure RO/NF membranes is an important operational tool to control biofouling. Quantitative information on the efficacy of cleaning agents and protocols to remove biomass is scarce. Therefore, a laboratory cleaning test to assess the efficiency of cleaning procedures to remove attached biomass was developed. The major components of the test are (i) production of uniform biofilm samples, (ii) the quantification of the biomass concentrations with robust parameters and (iii) a simple test procedure with optimal exposure of the biofilm samples to the chemicals. The results showed that PVC-P is a suitable substratum for the production of uniform biofilm samples. ATP and carbohydrates (CH) as major components of the biofilm matrix for nucleotides (living bacterial cells) and extracellular polymeric substances EPS, respectively, were selected as robust biomass parameters. The removal of ATP and CH with the NaOH/Sodium Dodecyl Sulfate (SDS) mixture, selected as a standard treatment at pH 12.0, was reproducible. The resistance of the EPS matrix against chemical cleaning was demonstrated by a low CH removal (32.8 ± 6.0%) compared to the ATP removal (70.5 ± 15.1%). The inverse relationship of biomass removal with the CH to ATP ratio (μg/ng) of the biofilms demonstrated the influence of the biomass characteristics on cleaning. None of the 27 chemicals tested (analytical-grade and commercial brands) in single step or in double-step treatments were significantly more effective than NaOH/SDS. Oxidizing agents NaOCl and H_2O_2, the latter in combination with SDS, both tested as common agents in biofilm control, showed a significantly higher efficiency (70%) to remove biofilms. In the test, simultaneously, the efficiency of agents to remove precipitated minerals such as Fe can be assessed. Validation tests with Cleaning in Place (CIP) in 8 and 2.5-inch RO membrane pilot plant experiments showed similar ranking of the cleaning efficiency of cleaning protocols as determined in the laboratory tests. Further studies with the laboratory test are required to study the effect of cleaning conditions such as duration, temperature, shear forces as well as chemical conditions (concentrations, alternative agents or mixtures and sequence of application) on the efficiency to remove attached biomass.

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1. Introduction

High pressure spiral-wound membranes, nanofiltration (NF) or reverse osmosis (RO), are being used increasingly in the water industry over the past decade for desalination of seawater, reclamation of wastewater, production of industrial waters and treatment of surface water or groundwater for drinking water production. A major impediment in the operation of these membranes is biofouling (Flemming, 1997; Khedr, 2000; Ridgway and Flemming, 1996; Schippers et al., 2004), which increases energy consumption and production losses due to clogging of the feed channel and fouling of the membrane. Membrane biofouling is controlled by balancing pre-treatment of the feed water and membrane cleaning practices. Membrane cleaning is an important control measure in membrane filtration, because (i) biofouling occurs at the level of micrograms of nutrients (Hijnen et al., 2009), (ii) a certain degree of fouling of the membranes is inevitable (Ang et al., 2006) and (iii) pre-treatment is more expensive than membrane cleaning (Schäfer et al., 2001; Schippers et al., 2004; van der Kooij et al., 2010). Fouling of low pressure membranes is controlled by intensive cleaning procedures, but these are not applicable to high pressure RO/NF membranes, which require a mild cleaning regime (agents, concentrations and frequency). Frequent cleaning contributes to loss of membrane integrity, shortening of lifetime and increased operational costs (Schippers et al., 2004; Trägårdh, 1989), as well as undesired environmental effects (Latteman, 2010). Consequently, optimizing cleaning procedures is beneficial in the management of spiral-wound membrane filtration.

The cleaning efficacy depends on several conditions, such as the membrane and fouling type, timing of cleaning, the choice of cleaning agents and also the procedural cleaning conditions (e.g. temperature, pH, duration, mechanical shear). Alkaline and acidic agents, detergents, complexing, biocidal and denaturizing chemicals are being used individually, or in combination in one or multiple step treatments with different sequences. Studies in literature, generally, focused on the effects of cleaning on the recovery of flux and pressure drop (van der Kooij et al., 2010). Few studies investigated the effect of cleaning on attached biomass systematically (Bereschenko et al., 2011; Chen and Stewart, 2000; Corpe, 1974; Whittaker et al., 1984), using different analytical techniques (suspended solids, microscopy, microbial and biochemical analysis). But with few quantitative results, thus, there is still a lack of scientific and quantitative knowledge on the efficiency of cleaning procedures for high pressure membranes fouled with attached biomass. Due to the complex and variable conditions of the cleaning procedures extended studies in practice are not feasible. Therefore, the applied procedures are tailor-made, based on trial and error at local conditions with variable fouling layers, and/or include the use of commercial products of unknown composition.

Given the importance of cleaning procedures for stable operational membrane performances and the central role of biomaterials in fouling of high pressure membranes (Schaule et al., 1993; Villacorte et al., 2009; Mondamert et al., 2011), there is a need for a cost-effective test for ranking cleaning procedures for their efficacy to remove attached biomass. Preliminary studies demonstrated the potential of a laboratory test using PVC-P biofilm samples (van der Kooij et al., 2011). Biomass removal was measured by using the parameters adenosine triphosphate (ATP) and carbohydrates (CH), which are well-suited for quantitative assessment of biofouling in high pressure membranes (Hijnen et al., 2011). Removal of bacterial ATP exhibits the impact of cleaning on the active part of the attached biomass, and CH removal represents the effects on the extracellular polymeric substances (EPS). Removal efficiencies observed in the preliminary tests and during Cleaning In Place (CIP) of intact spiral-wound membranes with similar cleaning protocols, were in the same order of magnitude, but showed a high level of variability (van der Kooij et al., 2011).

The objective of the current study is to optimize the laboratory cleaning test and, subsequently, to apply the test on a range of cleaning agents used in RO/NF-membrane practices, in order to rank cleaning efficiencies. In an additional pilot plant experiment with spiral-wound membrane elements the validity of this ranking was tested. The optimization of the laboratory test included the production of uniform biofilm samples under well-defined hydraulic conditions, comparative to the flow conditions in high pressure membranes, and the measurement of removal of attached biomass from these samples, before and after exposure to different chemicals under standardized conditions.

2. Materials and methods

2.1. *Production of uniform biofilm samples*

Uniform biofilm samples were produced by using plasticized polyvinylchloride (PVC-P) as a substratum, because of its high biofilm formation potential (van der Kooij and Veenendaal, 2001). Cylindrical biofilm samples (CBS) were produced from PVC-P tubing (Emergo, ∅ 12 mm) and flat biofilm samples (FBS) were produced from PVC-P plates from PVC-P sheets (Eriks, Mipolam 3 mm). The PVC-P material was incubated in a water recirculation system using (i) 55 cm PVC-P tubing (Cylindrical Biofilm Sample (CBS1) System), (ii) 20 PVC-P tubing pieces of 2.5 cm stacked in a glass column of 68 cm with an inner diameter of 2.8 cm (CBS2) and (iii) PVC-P plates of 2.5/2.5 cm positioned in five parallel rows of 13 plates in series in a hard PVC reactor of 4.2/2.5 cm (width/height; Flat Biofilm Sample (FBS) system). The production systems are schematically depicted in Fig. 1 and were supplied with non-chlorinated tap water with replenishment of fresh tap water to avoid oxygen and nutrient limitation. The applied flow rates in the production systems are presented in Table 1 with all the other detailed process conditions. These flow rates were in the same order of magnitude as in spiral-wound membranes, to produce biofilms under appropriate hydraulic flow conditions (Dow, 2008). The FBS system was operated with pre-filtered (1.2 μm) surface water during the first 24 h, to inoculate the system with a biodiversity of bacteria. Moreover, the FBS system was supplied with additional nitrate-N and phosphate-P to avoid nutrient limitation (Table 1).

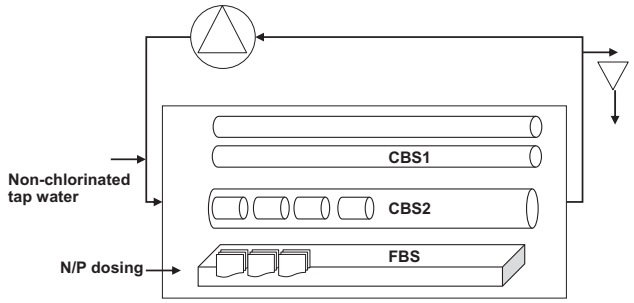

Fig. 1 – Schematic presentation of the biofilm recirculation systems.

2.2. Biofilm analytical methods

For determining the concentration of attached biomass, the biofilm on the PVC-P surfaces was removed and suspended in MilliQ water. For this purpose, PVC-P samples were brushed in 40 ml of Milli-Q water for 2 min using an electric toothbrush (Braun) with an autoclaved and cleaned tip. The obtained suspension with tip was sonified (High Energy Sonication Branson W-250 with sonotrode tip Ø 6.5 mm) for 5 min at 45% amplitude to homogenize the biomass. Subsequently, adenosine triphosphate (ATP) and carbohydrates (CH) was measured and the concentration of active biomass (ng ATP/cm^2) and extracellular polysaccharides of the biofilm (EPS; μg CH of glucose equivalent/cm^2) was calculated as previously described (Hijnen et al., 2011). For the CH analysis with the Dubois method (Dubois et al., 1956) the biofilm suspension samples were diluted when necessary with Milli-Q water to obtain concentrations at or below the maximum concentration of the standard calibration curve (30 mg glucose/l). Both parameters were analyzed in duplicate, giving a relative standard deviation of 3.7 ± 4.3% for ATP and 3.1 ± 6.3% for CH, respectively. Based on the detection limit for the ATP analysis (1 ng/l) and the CH analysis (1 mg/l), and the sample size of the substratum, the level of detection in the biofilm samples was 2–3 pg ATP/cm^2 and 4 μg CH/cm^2, respectively. Measuring of the biofilm concentration on the same PVC-P sample with three separate brushing and HES treatments in series showed that the recovery of this method was high (97.8 ± 0.8 and 92.5 ± 2.3% for ATP and CH, respectively).

Table 1 – Process conditions of the biofilm sample production systems.

	CBS	FBS
PVC (diameter D; Height/width H/W; mm)	D = 12	H/W = 25/25
Surface area biofilm samples (cm^2)	9.4	12.5
Flow reactor dimensions (ID tube; W/H plate; mm)	12; 28	42/25
Cross section area reactor (×10^{-4} m^2)	1.13; 5.75[a]	0.0008
Recirculation flow (l/h)	150; 160[a]	200; 350[b]
Water volume of the system (l)	26; 0.8	3.6
Surface to volume ratio (m^2/m^3)	333; 167	236
Tap water flow (l/h)	32; 0.13	20[c]
Flow rate (m/s)	0.37; 0.1	0.12
Reynolds number	4900; 3049[a]	695; 1217[b]
Temperature (°C)	23; 25[a]	22; 25[b]
Fe (mg/l)	0.34	0.06[c]
Phosphate-P (mg/l)	0.034	0.38[d]
Nitrate-N (mg/l)	0.12	0.85[d]

a CBS1; CBS2.
b FBS1; FBS2.
c Pre-filtered (10 μm poly-propylene cartridge filtration; Van Borselen Ltd.).
d Additional dosing.

2.3. Laboratory test protocol for chemical cleaning

PVC-P biofilm samples were placed in 200 ml MilliQ water (reference samples) and 200 ml chemical solution in MilliQ water (treated samples) in 600 ml beakers. CBS samples (tubing) were tested in triplicate by cutting the samples longitudinally, placing one part freely in the MilliQ water (reference sample) and the other part freely in the chemical MilliQ solution (treated samples). Three lined up FBS samples were placed upright in a holder in the beakers, one in the MilliQ water (reference sample) and two in the chemical MilliQ solution (treated samples). The beakers were placed on an orbital shaker (IKA 20 – orbital diameter = 30 mm) and agitated at 100 rpm for 1 h at 20 °C. The biofilm samples from the chemical solution were placed in 200 ml MilliQ water for 15 min under the same conditions, to remove the chemicals (rinsing). For double-step treatments, both steps with different chemicals were performed successively, under identical conditions. These double-step treatments were performed in an incubator at 35 °C (Snijder Scientific). After rinsing of the treated samples, the reference and treated samples were analyzed for biofilm concentration. The cleaning efficiency (CE; %) is calculated from the difference between ATP and CH concentrations on the reference and the treated PVC-P samples, respectively.

2.4. Chemical cleaning of RO modules

Three 2.5 inch RO membrane elements (TW30-2540 from Dow Filmtec) were operated in a pilot plant, which was described by Cornelissen et al. (2007). The installation was supplied with prefiltered (1 μm) chlorine free tap water at 0.35 m^3/h supplemented with 10 μg acetate-C/l, to induce biofouling. The process was operated at constant feed pressure, 0.055–0.075 m^3/h permeate production (recovery of about 20%) and temperature of 10–13 °C. The pressure drop development in the elements was measured daily. After 85 days, element 2 was cleaned by 1 h soaking with NaOH pH12 and SDS 1% (standard treatment in laboratory test), followed by 30 min flushing with feed water at 0.35 m^3/h. Element 3 was flushed for 1 h with Divos 2 (0.4 wt%; 35 °C, 0.35 m^3/h), followed by 30 min of water flushing at 20 °C at the same flow rate. Subsequently, the element was soaked with Divos 116 (8.8 wt%, 35 °C), followed again by 30 min of water flushing. The three elements were sampled at the inlet, middle and outlet for assessment of attached biomass as previously described (Hijnen et al., 2011).

2.5. Chemicals used

Different chemicals were tested, in single step treatment or in double-step treatments (Table 2). A major selection criterion for the applied conditions (pH and concentrations) is the compatibility with RO/NF membranes. The concentrations and pH values applied for the different chemicals were derived from field data. The selected double-step protocols are currently applied at a number of water supply companies in the Netherlands (references in the Acknowledgments) using high pressure membrane processes.

2.6. Hydraulic calculations

The hydraulic conditions in the biofilm production units were characterized by the Reynolds number in the flow chambers

$$\mathrm{Re} = \frac{\rho \cdot v \cdot d_h}{\mu} \quad (1)$$

where ρ is water density at 25 °C (997.0479 kg/m^3), v the velocity based on the actual cross section area of the flow channel or pipe (m/s) calculated from the flow rate (m^3/s) and the cross section area (m^2), μ the viscosity at 25 °C (9.00×10^{-4} N s/m^2) and d_h the hydraulic diameter (m). For the PVC-P tubing d_h was calculated with the inner diameter ID of 1.2 cm and for the rectangular flow channels between the rows of PVC-P plates d_h is calculated from

$$d_h = \frac{4 * WH}{2 * (W + H)} \quad (2)$$

The range of possible flow rates in the laboratory test was calculated from the diameter of the beaker (0.10 m), the revolutions per minute during the test of 100 and the placement in the beaker at 0.005–0.03 m from the central axis. This yields maximum flow rates of 0.026–0.16 m/s which is in the range of the flows in the feed channel of spiral-wound membranes (0.1–0.2 m/s, Dow, 2008). Since the fluid will also flow in the same direction and local flow rates in the feed spacer will be higher due to the presence of a spacer, shear at the conditions in the laboratory test is assumed to be lower than shear in an element during flushing actions in a CIP.

2.7. Statistical calculations

Statistical analysis of the data was performed with SPPS 17.0. Normal distribution and equality of variances were tested with the Kolmogorov–Smirnov test ($P > 0.05$) and the Levene's test ($P > 0.05$) and comparison of means using the student-t test ($P < 0.01$). The cleaning test results were statistically tested by the Anova analysis and the Fisher LSD or Bonferoni test ($P < 0.05$). Correlations were tested with Pearson (linear) and Spearmen rho (non-linear) coefficients for paired ATP and CH removal, and the pH at the different tests.

3. Results

3.1. Production of uniform biofilm samples

The production of cylindrical (CBS) and flat (FBS) biofilm samples (CBS and FBS systems) was established within a 40–60 days period in which the ATP and CH concentrations increased (Fig. 2), and a biofilm was observed. Instability

Table 2 – Cleaning chemicals (Suppliers) and their applied conditions arranged according to the applied categories.

Category (supplier)	Concentration (%; w/v or M); pH	Category (supplier)	Concentration (%; w/v or M); pH
Alkaline agents:		*Acids*:	
NaOH (Boom chemicals)	0.04-1; 12-12.7	Citric (Boom chemicals)	1; 2
Floclean MC11 (BWA)[a]	2; 11	HCl (Boom chemicals)	0.01 M; 2
Permaclean PC-33 (Nalco)	2; 12	Divos 2 (JohnsonDiversey)	0.4; 1.6
Novoclean 135 (Novosystems)	0.4; 11.6	Permaclean PC-77 (Nalco)	4; 4
Divos 116 (JohnsonDiversey)	0.8; 12	P3-ultrasil 73 (Ecolab)	1; 2.5
P3-ultrasil 141 (Ecolab)	0.1; 11		
Detergents:		*Biocides*:	
Na Dodecyl Sulfate (Boom)	1; 8	DBNPA (Sigma-Aldrich)	0.005; 6
Na Triphosphate (Sigma-Aldrich)	2; 9	Na Bisulfite (Brenntag)	0.05; 4
Trisodium Phosphate (Merck)	1; 12	NaOCl (Boom) + NaOH	0.5; 11.4
CTAB (Boom)	1; 5	P3-oxyzan ZS (Ecolab)	0.1; 3
NaDDBS (Sigma-Aldrich)	0.025; 7	H_2O_2 (J.T.Baker) +/− NaOH	0.5; 7, 11
Enzymes:		*Enzymes*:	
P3-ultrasil 53 (Ecolab)	1; 9	Dextrozyme (Novozymes)	0.1; 5
BAN 480 (Novozymes)	0.1; 5	Savinase (Novozymes)	0.1; 7
Chelating agents:		Everlase (Novozymes)	0.1; 7
EDTA (Boom)	0.01 M; 11		
Double-step low − high pH:		*Double-step high − low pH*:	
Citric+NaOH	2; 12	NaOH/SDS + HCl	0.04–0.02; 12–2
Divos 2+Divos 116	2; 12	NaOH/HCl	0.04–0.01M; 12–2
Citric+Novoclean 135	2; 12	NaOH/Divos 2	0.04–0.4; 12–2
		PC33+PC77	2–4; 12–4
		Na-Bisulfite/NaOH+Divos 2	0.05/0.04–0.4; 12–2

a Citric, TSP, STP, EDTA mixture.

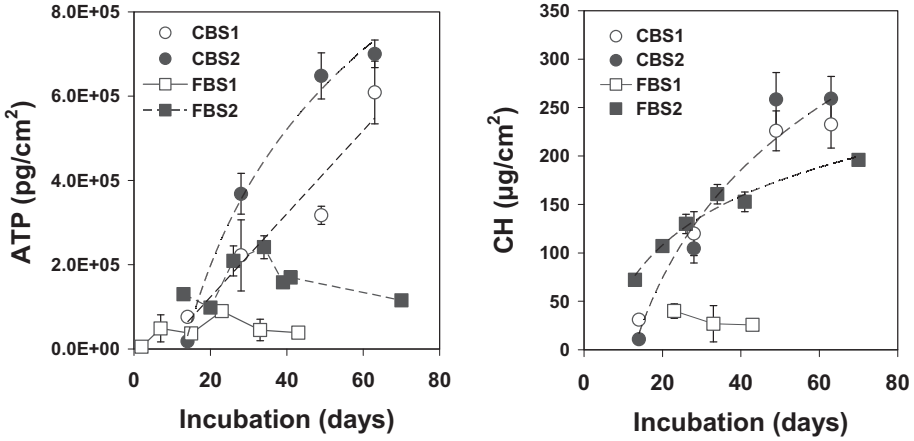

Fig. 2 – Biofilm growth (ATP and CH concentrations with standard deviation SD presented by the error bars; $n = 2$) on PVC-P tubing or PVC-P plates in the CBS and FBS recirculation systems.

observed in the CBS system (some CBS biofilms were unfit for testing; data not presented) and optimization of the cleaning test conditions, were the rationales to include the production system with flat PVC-P material (Table 1). The biofilm grown in the CBS systems (CBS1 and CBS2) showed higher ATP and CH concentrations than on the PVC-P of the FBS system. CBS samples were harvested for cleaning tests during the period between day 49 and day 63. Under the low flow conditions in the FBS1 (200 l/h; Table 1), biofilm production was too low (Fig. 2). At the increased flow conditions (FBS2), ATP and CH production reached to higher and stable levels, fit for tests. FBS samples for cleaning tests were harvested between day 26 and 43 with average ATP and CH concentrations, which were lower than on the CBS samples (Table 1; supplementary information). The CH/ATP ratio (μg/ng) is a characterization of the biofilm structure, and was not similar in the two biofilm production units. The ratios of the samples from the two CBS production units (CBS1 and CBS2) were not significantly different ($P = 0.37$) with an average ratio of 0.46 ± 0.14 μg/ng. The average CH/ATP ratio for the FBS biofilms samples was 0.77 ± 0.21 μg/ng, and significantly ($P < 0.01$) higher than the CH/ATP ratios of the CBS biofilms samples.

3.2. *Biofilm removal by chemical cleaning: influence of the biofilm matrix*

The reference biofilm samples used to calculate the cleaning efficiencies of the cleaning agents were, simultaneously, agitated on the orbital shaker at 100 rpm treated in MilliQ water. To verify the effect of agitation on the biofilm, ATP and CH concentrations of untreated and reference biofilm samples were compared. The results, presented in Table 1 of the supplementary information, revealed that the agitation during the laboratory test was not responsible for biomass loss. The average ATP and CH concentrations of all untreated and reference samples were not significantly different. Thus, the observed biomass loss in the tests with chemicals was caused by the chemicals in combination with agitation.

The effect of chemical cleaning in the test protocol and the reproducibility of this effect, was assessed with a standard treatment using a mixture of NaOH and SDS (Sodium Dodecyl Sulfate; 1%), first at pH12.7 for the CBS samples and, subsequently, for the FBS samples at a pH of 12.0 to test under the more common pH conditions used in practice. The removal of CH from the CBS samples at pH12.7 was 59.7 ± 11% (black squares; Fig. 3a) and clearly lower than the removal of ATP from the same samples >99% or 2.9 ± 0.3 log ($n = 18$; black squares Fig. 3b). The removal of CH and ATP was significantly ($P < 0.001$) higher for the CBS biofilm samples than for the FBS biofilm samples ($n = 19$; gray symbols Fig. 3a, b). To determine the effect of pH, biofilm removal from the FBS samples by the NaOH/SDS was also tested at pH 12.7 ($n = 4$). The pH had hardly any effect on the removal. At pH 12.7, the removal of ATP of 1.5 ± 0.8 log and of CH of 37.8 ± 11.1% ($n = 4$; gray triangles in Fig. 3a, b) was not significantly different from the removal of both components from the FBS samples at pH12.0 ($P > 0.05$; $n = 10$; gray circles in Fig. 3a, b). Thus, the difference in cleaning effect of the standard treatment NaOH/SDS on the CBS and FBS biofilms, was caused most likely by another factor. Difference in the characteristics of the produced PVC biofilms is such a factor. The exponential relationship between CH/ATP ratio and the removal of CH ($P < 0.001$) and ATP ($P < 0.001$) depicted in Fig. 3, showed that the largest removal was observed at ratios ≤0.5 μg CH/ng ATP. From the presented curves it was concluded that for an objective comparative assessment of cleaning efficiency (CE) of different cleaning agents and procedures, the tests must be executed with PVC-P biofilm samples at CH/ATP ratios of >0.8. To account for the effect of the biofilm matrix on the CE, the treatment NaOH/SDS at pH 12.0 is introduced in every cleaning test to assess the relative differences in cleaning efficiency.

3.3. *The cleaning efficiency of individual chemicals*

The effect of the treatment with the mixture of NaOH/SDS (1%; pH 12.7) was compared with the effect of the treatments with NaOH and SDS, separately, in two tests with CBS samples. High ATP removal (≥90%) was observed in the treatments at high pH with either the mixture NaOH/SDS or NaOH, whereas ATP removal with SDS at pH 8.0 was 33.1% on average (Table 2; supplementary information). Removal of EPS

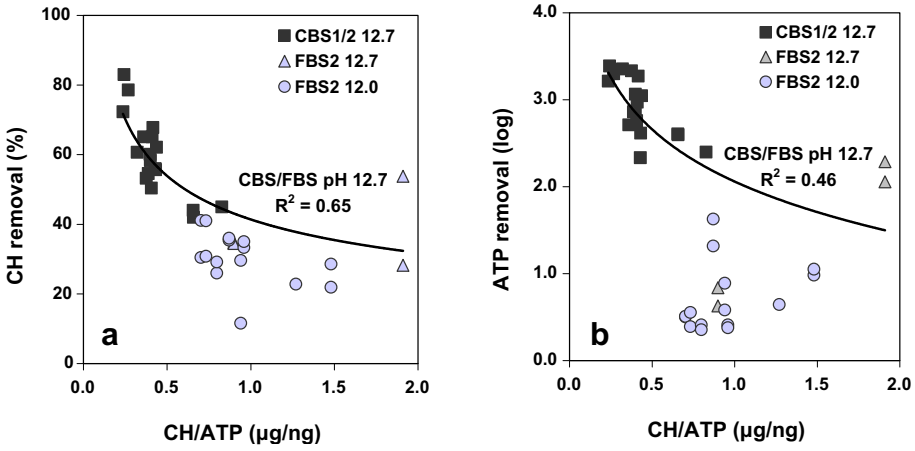

Fig. 3 – The cleaning efficiency (CE ± SD) of the standard test with NaOH/SDS at two pH values, using biofilm samples from the CBS and FBS production systems and correlated with the CH/ATP ratio (µg/ng; ±SD) of the tested PVC-P biofilms.

(CH) was less efficient than the removal of ATP. CH removal (52.7 and 57.0%) was significantly higher ($P < 0.001$) with the mixture NaOH/SDS than observed in the tests with the individual chemicals NaOH and SDS. The sum of the average values for the CH removal by the separate treatments with NaOH and SDS was 45.5% and lower than the average of the CH removal 54.9 ± 8.0% ($n = 12$) observed for the mixture NaOH/SDS, suggesting that the use of both chemicals in a mixture has a synergistic effect on biofilm removal. A similar conclusion was presented for cleaning of ultrafiltration membranes (Li et al., 2005).

A number of individual chemicals (analytical-grade chemicals and commercial brands; Table 2) were tested with the laboratory cleaning test protocol at 20 °C, using FBS samples and NaOH/SDS as the standard treatment. The cleaning efficiencies for CH and ATP removal are presented in Fig. 4 for four categories: alkalines, acids, detergents and a combined group with biocides, EDTA as a chelating agent, and dextrozyme as an enzyme. The average cleaning efficiencies assessed for CH and ATP show that the chemicals could be distinguished according to their cleaning efficiency. In each group significant differences ($P < 0.05$) were observed

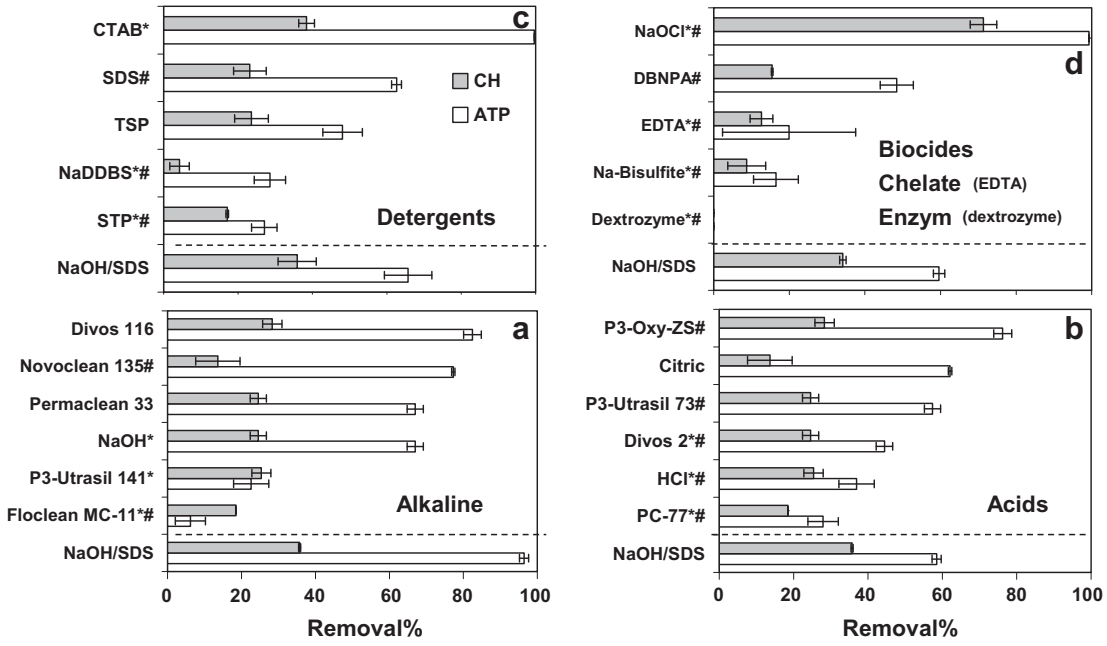

Fig. 4 – The cleaning efficiency (CE; ATP, CH removal %) of the individual chemicals in a one step treatment at 20 °C (error bars range of duplicates; * and # indicate significantly different ($P < 0.05$) from standard treatment for ATP and CH removal, respectively).

between the tested chemicals and the standard treatment with NaOH/SDS.

The standard treatment in the different tests showed an average ATP removal of 69.8 ± 15.0%, and CH removal of 33.8 ± 4.9% ($n = 10$; CH/ATP ratio of 0.8 ± 0.1). Generally, the ATP removal was clearly larger than the CH removal, and showed a higher variability between the chemicals with efficiencies ranging from 0 up to ≥99%. The CH removal observed in the different tests showed also distinct differences between the different agents (0–70%), but the variability was less than observed for ATP. The investigated acids and alkalines (analytical-grade and commercial brands) showed for most agents lower ATP and CH removal than the standard treatment (Fig. 4a, b). The commercial agents, which are unknown blends, showed similar or sometimes lower biofilm removal effects than the analytical-grade agents (NaOH, citric acid and HCl). In the group of detergents, the only tested cationic detergent CTAB (Hexadecyltrimethyl-ammonium bromide; positively charged at pH 5.0) was significantly more effective in ATP removal than the standard treatment (Fig. 4c). The anionic detergents SDS, STP (Sodium Triphosphate), TSP (trisodium phosphate) and NaDBBs (sodium dodecylbenzenesulfonate) at neutral pH or pH 12.0 (TSP), showed lower removal of ATP and CH than the standard treatment. In the group of biocides, the oxidizing biocide NaOCl (sodium hypochlorite; 5 g/l in combination with NaOH at pH 11.4), removed CH from the PVC-P surface for 68 and 75% with an ATP removal of >99% (Fig. 4d). The other biocides, DBNPA (2,2-Dibromo-3-nitrilopropionamide) and sodium bi-sulphite, and the chelating agent EDTA (pH11), and the enzyme Dextrozyme (glucoamylase; pH5), had low cleaning efficiencies. Treatment with Dextrozyme and the other enzymes (BAN 480; alpha-amylase; Savinase and Everlase; proteases) increased the ATP and CH concentrations; a preceding NaOH/SDS treatment before BAN480 and Savinase, did not enhance the ATP and CH removal by these enzymes (data not presented). Thus, the enzymes apparently raised the metabolic activity in the biofilms, and no biofilm removal was observed. The CH increase suggests that the enzymes accumulated in the biofilm and raised the results of the CH analysis (Dubois method). In conclusion, these tests showed that the removal of EPS (CH) was usually lower than 40% and only a few chemicals viz. NaOCl performed better than the standard treatment NaOH/SDS. A milder oxidizing agent, hydrogen peroxide H_2O_2 was tested separately at neutral pH and high pH (combination with NaOH), and in combination with NaOH/SDS at pH 11. This biocide showed low cleaning efficiency at neutral pH and increased efficiency at pH 11 (Fig. 5). The efficiency of peroxide reached an efficiency of 72.3 ± 2.3%, as observed for NaOCl, when it was used in a mixture with NaOH (pH11) and 1% of SDS.

3.4. *Cleaning efficiency of double-step treatments*

A number of agents were also tested in double-step treatment in the sequences and concentrations, applied in membrane cleaning practices at full-scale. In these treatments the sequence of the acid and alkaline treatment varied. All the double-step tests and also the standard test with NaOH/SDS pH 12, were conducted at an elevated temperature of 35 °C as done at full-scale. The ATP and CH removal by the NaOH/SDS treatment in these tests were 85.4 ± 7.9 and 22.9 ± 8.3%, respectively (Table 3; $n = 4$). This average ATP removal was higher than observed for the reference test at 20 °C, but the difference was not significant ($P > 0.05$). The CH removal by NaOH/SDS at 35 °C was significantly ($P < 0.05$) lower than at 20 °C, which coincided with a higher CH/ATP ratio (Table 3).

The double-step treatments are categorized in Table 3 according to the sequence in pH, with the sequence of high – low pH first. ATP removal of seven out of eight double-step treatments was significantly lower than the ATP removal in the standard treatment, except for the combination of citric acid with Novoclean 135. Also the CH removal data showed that most double-step treatments did not remove EPS more efficiently than the standard treatment (Table 3). The only treatment with a significantly higher CH removal than the standard treatment was the acid – alkaline treatment with Divos 2 and Divos 116, despite the low ATP removal for this

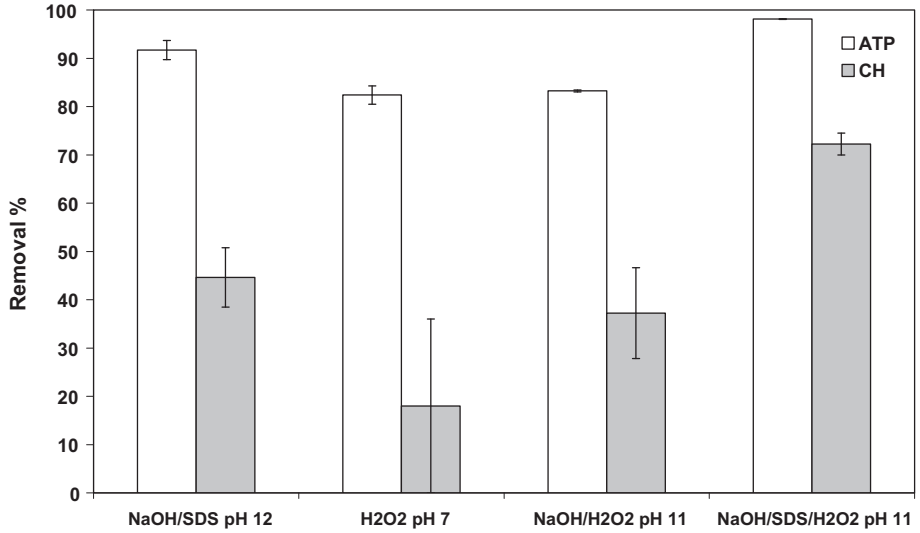

Fig. 5 – ATP and CH removal by the standard cleaning test and by hydrogen peroxide under different conditions.

Table 3 – The average cleaning efficiency of the double-step treatments at 35 °C applied at Dutch RO/NF membrane filtration plants (* significantly different from standard treatment NaOH/SDS; $P < 0.05$).

Treatment step 1 + step 2	pH	ATP ± SD %	CH ± SD %
NaOH/SDS 20 °C ($n = 10$; CH/ATP 0.8)[a]	12.0	69.8 ± 15.0	33.8 ± 4.9
NaOH/SDS 35 °C ($n = 4$; CH/ATP 1.2)[a]	12.0	85.4 ± 7.9	22.9 ± 8.3
PC-33 + PC-77[b]	11.8 + 3.9	12.1 ± 7.4*	10 ± 2.0*
Bisulfite/NaOH + Divos 2[b]	11.7 + 1.8	31.2 ± 6.9*	30.4 ± 3.4
NaOH + HCl[b]	12.0 + 2.0	47.1 ± 0.3*	21.5 ± 1.0
NaOH + Divos 2[b]	12.0 + 1.8	48.8 ± 10.5*	25.9 ± 3.7
NaOH/SDS + HCl[b]	12.0 + 2.0	58.5 ± 6.1*	29.6 ± 6.8
Citric + NaOH[b]	2.0 + 12.0	25.3 ± 2.9*	25.5 ± 1.2
Divos 2 + Divos 116[b]	1.8 + 12.2	32.0 ± 6.1*	50.3 ± 1.9*
Citric + Novoclean 135[b]	2.0 + 11.9	93.3 ± 1.1	23.8 ± 1.5

a Standard treatment at 20 °C and 35 °C.
b Double-step treatments (Table 2).

treatment. Significantly lower than the standard treatment was the CH removal efficiency by PC33 and PC77 treatment. Thus, under the applied test condition, most double-step treatments at 35 °C did not result in more removal of attached biomass than a one step treatment with NaOH/SDS at 20 °C.

3.5. *Cleaning-in-place of fouled membrane elements*

The predictive value of the ranking of the chemicals, based on the laboratory test for Cleaning in Place (CIP) procedures of spiral-wound membranes, was first demonstrated in a cleaning experiment with a test rig pilot plant (Rapenne et al., 2011). Similar chemical cleaning protocols were tested in this plant with 8 inch biofouled elements from a full-scale desalination plant (van der Kooij et al., 2011) and in the current laboratory test. The average ATP and CH removal efficiencies and ranking, assessed under both conditions, were in the same order of magnitude (Table 4). However, the variation in the pilot plant results was high (relative SD of 10, 50, 65 and >100%).

In the current study, a similar comparative test was performed in a pilot plant experiments with 2.5 inch membrane elements. After an operational period of 85 days, where three elements were supplied with acetate enriched tap water (10 μg C/l), a pressure drop increase ($dP_i = dP_o - dP_{end}$) of 0.4–0.6 Bar was reached (Fig. 6). The exponential fouling rate constant R_f, calculated from these data, ranged from 0.064 to 0.084 d^{-1} (10–13 °C). This was in the same order of magnitude as observed at acetate concentrations of 5 and 10 μg C/l (0.062–0.147 d^{-1}) at 16–19 °C in previously published biofouling experiments (Hijnen et al., 2009). This confirms that the modules were fouled by biofilm.

Elements 2 and 3 were tested with the standard treatment NaOH/SDS pH 12 and the most effective procedure of the double-step treatments Divos 2 and Divos 116. The reduction of ATP and CH concentrations in the membrane elements are depicted in Fig. 6. The cleaning efficiencies calculated from these data showed that the efficiency in the CIP of the elements was higher than observed in the laboratory test (Table 4). However, as observed in the test described before, the ranking under both conditions (pilot plant and laboratory test) was similar, and also the relative difference in cleaning efficiency of both cleaning treatments, assessed under both conditions, was similar, thus, indicating the predictive value of the laboratory test.

4. Discussion

4.1. *Biofilm quantification: biomass parameters*

Biofilms are composed of active bacterial cells, EPS and (in) organic components (Flemming and Wingender, 2010). The biomass parameters ATP and carbohydrates (CH) measured with the Dubois method (Dubois et al., 1956) correlated with the increase of pressure drop in the feed water channel of high pressure membranes (Hijnen et al., 2011). The nucleotide adenosine triphosphate (ATP) is present in all living bacterial cells (Karl, 1980). Bacterial EPS is of particular importance in

Table 4 – Comparison of the biomass removal (%±SD, $n = 2$) determined in the laboratory test with PVC-P biofilms with the biomass removal determined in two CIP pilot plant studies with biofilms samples[a] from an 8 inch RO element of a full-scale desalination and with biofilm samples[c] from a 2.5 inch RO element operated in a pilot plant at KWR.

	CIP RO-membrane		Laboratory test	
	ATP	CH	ATP	CH
Biomass (ng/cm^2; $\mu g/cm^2$)	*1.0–1.6*[a]	*79.3–142.3*[a]	*83.0–145.5*[b]	*104.2–132.9*[b]
NaOH – HCl (CE%)	48.0 ± 4.5	15.3 ± 36.9	47.1 ± 0.3	21.5 ± 1.0
NaOH/SDS – oxalic acid[a]/HCl[b] (CE%)	68.0 ± 33.4	43.6 ± 30.2	58.5 ± 6.1	29.6 ± 6.8
Biomass (ng/cm^2; $\mu g/cm^2$)	*4.1–40.0*[c]	*16.1–59.3*[c]	*93.2–85.7*[b]	*88.2–87.7*[b]
NaOH/SDS pH 12 (CE%)	98.6 ± 0.9	54.2 ± 3.0	85.4 ± 7.9	22.9 ± 8.3
Divos 2/Divos 116 (CE%)	97.9 ± 0.4	76.8 ± 0.7	32.0 ± 6.2	50.3 ± 1.9

a 8 inch RO pilot plant, Rapenne et al., 2011, van der Kooij et al., 2011; CH/ATP ratio membrane biofilms ≥50.
b Optimized laboratory test Table 3.
c 2.5 inch pilot plant current study; CH/ATP ratio membrane biofilms >4.

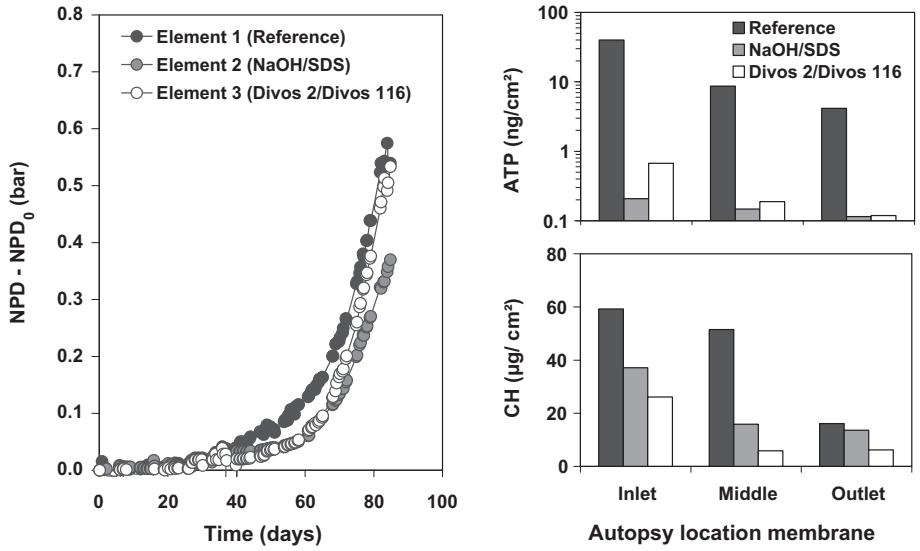

Fig. 6 – Pressure drop development in three separate 4 inch RO modules loaded with chlorine-free tap water, supplemented with 10 μg acetate-C/l (triplicates), and the attached biomass concentrations (ATP and CH) of the reference module and two chemical cleaned modules, at three specified locations.

cleaning, due to its protective nature against chemical stress (Strathmann et al., 2001). EPS is mainly composed of polysaccharides and proteins, with a major fraction of polysaccharides in EPS-rich bacterial strains. The negative charge of EPS has been demonstrated in literature (Tsuneda et al., 2003; Flemming et al., 2007; Wang et al., 2011). Below the pH of the iso-electric point, EPS is positively charged and more dense and compact. At high pH values, EPS is negatively charged and thereby repulsive, which results in weaker intra- and inter-colloid interactions and release of chains, swelling and lower density structures. Also the level of hydrophobicity influences biofilm attachment, and susceptibility to detachment during cleaning as presented before (Ridgway et al., 1985; Whittaker et al., 1984). Accumulation of mono- and multi-valent ions, such as Fe or Ca in biofilms, cross-linked to the ionic groups in the EPS affects the structure of biofilms (Li and Elimelech, 2005; Ang et al., 2006).

Biofilms were visually present on the CBS and FBS. Staudt et al. (2004) presented thicknesses of biofilms on polycarbonate slides of 100 μm. Assuming similar biofilm thicknesses on our FBS, an ATP content per cell of 3.6×10^{-7} ng ATP (Magic-Knezev and van der Kooij, 2004), a cell weight of 1×10^{-12} g and a wet density ρ of 1.0 g/ml, it can be estimated that the active bacterial cells measured with ATP fraction is approximately 2.8–5.6% of the wet biofilm volume, and the EPS fraction quantified with the Dubois method represents approximately 1.5% of this wet biofilm volume. Despite these low percentages, ATP and CH concentrations are correlated with pressure drop in membranes, as stated before. The threshold values for a relative pressure drop (PD) increase in the feed channel of 100%, is 3.7 ng ATP/cm^2 and 8.1 μg CH/cm^2, respectively (Hijnen et al., 2011). Reduction of the pressure drop to ≤10% relative PD increase, requires biomass removal to at least the detection limits of ATP of 1 ng and of CH of 5 μg/cm^2, which equals approximately 70% ATP and 50% CH removal. The volumetric reduction of the biofilms quantified in the current study by ATP and CH, was confirmed by additional studies with confocal laser scanning microscopy (CLSM; data not presented, Castillo et al. in preparation). The overall results in this study showed that CH removal by chemical stress is always lower than ATP removal. Despite this physiological difference between ATP and CH, the removal of these compounds for all chemical agents ($n = 74$) was significantly linearly correlated ($P < 0.01$). Because of the low R^2 of 0.40, and low regression coefficient of 0.34, however, cleaning efficiency must be assessed by both parameters separately.

4.2. *Uniform PVC-P biofilm samples as model for membrane biofilms*

Plasticized PVC in the tube (CBS) and on the plates (FBS), stimulated a significant biofilm growth in recirculation systems, supplied with non-disinfected drinking water at 25 °C. In these recirculation systems the objective was to produce uniform biofilm samples. The biomass concentrations assessed with ATP ranged from 2.4×10^5 on the plates (FBS), to 7×10^5 ATP/cm^2 on the tubing (CBS). This was in the same order of magnitude as determined previously for PVC-P in a static batch test in chlorine-free drinking water, where the biomass production was 2.1×10^5 pg ATP/cm^2 (van der Kooij and Veenendaal, 2001). The concentration of carbohydrates in the biofilms on PVC-P ranged from 100 to 260 μg/cm^2, and was still increasing after 70 days (Fig. 2). The difference in CH/ATP ratio between the CBS and FBS samples, shows that biofilm production and composition is influenced by the materials (PVC-P tubing versus PVP-P plate) and the growth conditions viz. hydraulic stress (Reynolds number in CBS > FBS; Table 1), Fe concentrations (CBS > FBS). To perform the cleaning tests with uniform biofilm samples, most comparative tests in the current study were performed with FBS biofilm samples, and a standard treatment with NaOH/SDS was introduced. ATP and CH concentrations on the FBS

biofilm samples were 10–20 ng and 100–150 μg per cm^2, respectively. These concentrations are in the same order of magnitude as observed in a membrane autopsy study, where these parameters were correlated with pressure drop in the feed channel (Hijnen et al., 2011).

4.3. *The influence of biofilm characteristics on cleaning efficiency*

The observed inverse influence of the CH/ATP ratio on the cleaning efficiency (lower efficiency at higher ratio; Fig. 3) presents evidence for the important role of carbohydrates (EPS) in the stress resistance of biofilms against cleaning. The effect of hydraulic stress during biofilm growth on its resistance to the detachment during cleaning, has recently been presented (Vrouwenvelder et al., 2010). However, the Reynolds numbers in the FBS system with the high CH/ATP ratios indicated more laminar conditions during biofilm growth (lower hydraulic stress), than in the more turbulent CBS system (Table 1) with lower CH/ATP ratios. The turbulence in the FBS system may be higher than calculated from the dimensions, because of the irregularities in the six flow channels of the system, formed by five rows of 13 lined up plates. The other possible explanation for the difference in CH/ATP ratio is the continuation of biofilm growth in the CBS system during the sampling period, whereas the ATP concentrations in the FBS indicated more steady state conditions in the biofilm samples (Fig. 2). This indicates lower nutrient concentrations in the flat PVC-P material, compared to the PVC-P tubing (no information available).

Furthermore, the CH/ATP ratio as an indicator for the fouling resistance of biofilms at values above 0.8 μg/ng, is possibly of less importance. This is confirmed by the cleaning data from the pilot plant CIP studies, where the CH/ATP ratio's observed in the membrane biofilm samples were much higher than 0.8 (Table 4). Biofilms will vary in their resistance against chemical and mechanical stress, based on the biofilm growth conditions (nutrients and shear), as well as, on its history (aging and exposure to cleaning). This emphasizes the importance of timing and frequency of cleaning. Elucidating of the effect of these conditions on the biofilm resistance against cleaning procedures requires further studies.

4.4. *Effect of chemicals on attached biomass*

As stated before, few quantitative literature data are available for comparison (Corpe, 1974; Whittaker et al., 1984; Chen and Stewart, 2000). Only the experimental set up of the latter study was comparable to the current study. The biofilm produced in a continuous flow annular (CFA) reactor, was quantified with proteins and colony count R2A. The protein removal ranged from 16 to 71%, which is in the same order of magnitude as observed for CH removal in the current study. Chen and Stewart ranked some individual chemicals as follows: NaOCl ≈ SDS > alkaline > EDTA > acid. This ranking, is to some degree similar to the ranking of the group of chemicals in the current study: NaOCl > SDS ≈ Alkalines ≈ Acids > EDTA.

The oxidizing biocide NaOCl is not used for spiral-wound membrane cleaning and tested in the current study as a positive control of an effective agent against biofilms. Despite its evidently high cleaning potential for (bio)fouled RO and ultra filtration membranes (Baker and Dudley, 1998; Zondervan and Roffel, 2007), the damaging effect of free chlorine on the thin-film composite membranes is a serious draw back (Buch et al., 2008). NaOCl was only tested in combination with NaOH at high pH, because under these conditions it is available as hypochlorite (OCl^-), which is more effective in removing biofilms than hypochlorous acid (HOCl; Characklis, 1990). Despite the faster decomposition of H_2O_2 at elevated pH, CH removal increased when pH was increased with NaOH from 7 to 11 (Fig. 5). A hydrogen peroxide-based detergent was successfully used for medical devices (Alfa and Jackson, 2001), and also the current study showed that the combination of 0.5% H_2O_2 with 1% SDS at pH 11 was highly efficient to remove biofilms. Testing of the compatibility of this alternative cleaning agent for thin film composite membranes in water treatment requires further studies.

When all results of the current study with the individual chemicals were accumulated, a significantly correlation ($P < 0.01$) was observed of the pH of the chemical solutions, with ATP and CH removal (Spearman rho R of 0.382 for ATP and of 0.648 for CH). Increased efficiency was observed under both acidic and alkaline conditions, which is in accordance with the results presented in literature (Chen and Stewart, 2000; Corpe, 1974; Li and Elimelech, 2005). However, the fitted quadratic model showed a low level of fit (R^2 of 0.15 and 0.30 respectively), indicating that also other physical/chemical conditions affect the removal.

4.5. *Effect of cleaning conditions in membranes*

The results showed that none of the chemicals tested, analytical-grade and commercial brands, were significantly more effective than the combined treatment with NaOH/SDS. Furthermore, the cleaning efficiency to remove attached biomass was limited; for EPS <50%. In membrane cleaning practices, two major questions are of importance: (i) when to clean and (ii) how to clean. Criteria for the answer on question one, were derived from a literature survey (van der Kooij et al., 2010): 15–50% pressure drop increase, 10–15% MTC and TMP reduction or 10% loss in salt rejection. The biofilms tested in the current study can be characterized as severe biofilms leading to higher percentage of pressure drop increase than 15–50%. This is based on the ATP and CH concentrations and the previously published relation with pressure drop increase in membranes (Hijnen et al., 2011). Most likely, the high resistance of the tested PVC-P biofilms against chemical cleaning is caused by the relative high CH concentrations in the biofilms as stated before with respect to the influence of the CH/ATP ratio.

For the answer to the second question 'how to clean', besides the choice of chemicals, also aspects such as pH, concentration of the chemicals, temperature, duration of the treatment and shear forces are important variables in the efficacy of cleaning. pH during cleaning, should be carefully selected based on the fouling layer. From the double-step treatments, no preferred sequence in pH treatment could be

selected; high – low or low- high pH for the removal of attached biofilms. Additionally, the results of the double-step experiments, revealed no enhanced biofilm removal under these conditions, even though the tests were carried out at higher temperature of 35 °C. This is an unexpected observation and needs further verification.

The test described in this study, was developed to compare and rank membrane cleaning agents and procedures for their efficacy to remove biofilms under well controlled conditions. The cleaning conditions in the laboratory test are obviously different from the CIP conditions in full-scale membrane elements. The biofilm accessibility in the laboratory test is higher than in the membrane modules, but shear might be lower as argued before (paragraph 2.6). In the preliminary validation tests the cleaning results from the laboratory test and CIP procedures with fouled RO modules using similar cleaning procedures, were comparable in order of magnitude, but too variable (van der Kooij et al., 2011). In the pilot plant test of the current study, variability of the cleaning data was reduced, and comparison between the data of the laboratory test and the CIP, revealed similar cleaning data efficiencies, with respect to the ranking of the tested chemicals (Table 4). This similar ranking, observed under both conditions, confirms the potential of the laboratory test to select the optimal cleaning procedure for the removal attached biomass from spiral-wound membranes. The cleaning efficiency assessed with the CIP in the 2.5 inch pilot plant study, however, was higher than observed in the laboratory test, most likely due to higher shear forces during CIP in the 2.5 inch membrane elements. This can be verified by comparing the cleaning efficiency in the laboratory test assessed with biofilm samples from membranes next to FBS samples. Hydraulic shear forces have been applied successfully in controlling normalized pressure drop in high pressure membrane elements, using air/water cleaning (Cornelissen et al., 2009, 2007). This measure requires vertical positioned elements, whereas most installations apply horizontal positioned elements. The success of A/W cleaning is attributed to the introduction of high shear stress and wall shear rates, but even under these hydraulic conditions the success of removal of attached bacteria depends on the characteristics of attached cells (Gomez-Suarez et al., 2001; Sharma et al., 2005).

The effect of these above mentioned cleaning conditions, can be studied with the laboratory cleaning test prior to further validation in specified CIP studies, to optimize cleaning efficacy. Optimization of cleaning protocols, however, needs a clear view on the quantitative relationship between removal of attached biomass and the optimization of the operational conditions of the membrane process: pressure drop, recovery and retention. For pressure drop such relationship has been established (Hijnen et al., 2011), but there is still a lack of information on the quantitative relation between attached biomass concentrations and the other operational conditions (flux, retention). Thus, next to further quantification of the effect of cleaning conditions on removal of attached biomass, more (quantitative) information should be collected on the current cleaning practices of full-scale spiral-wound membrane processes to specify further research on cleaning.

5. Conclusions

A laboratory test was developed, which enables the ranking and selection of optimal cleaning procedures for spiral-wound membranes based on the efficiency to remove attached biomass from surfaces. Uniform biofilm samples on PVC-P were produced with reproducible attached biomass concentrations. In a simple standard laboratory test the removal of ATP and CH can be quantified, reflecting the elimination (inactivation and/or removal) of active bacterial biomass and the EPS matrix of the biofilm. The CH/ATP ratio is a characteristic of the biofilm related to its susceptibility to chemical stress. At a CH/ATP ratio of 0.8 μg/ng or less biofilms are more susceptible to cleaning. At higher ratios, cleaning efficiencies are lower and the effect of this ratio on the cleaning efficiency diminishes. Besides the removal of attached biomass, also the efficiency to remove inorganic substances, such as iron, can be quantified in the test.

With the test distinct differences in cleaning efficiencies between chemicals, analytical-grades and commercial brands, were observed. ATP removal was higher than CH removal which was usually ≤40%. The highest and reproducible efficiency, was observed for the standard procedure with NaOH/SDS (70% ATP and 34% CH on an average). Double-step treatments at 35 °C did not enhance biomass removal. The two tested oxidative agents, NaOCl and the milder H_2O_2 in combination with SDS, both at pH 11, showed significant higher CH removal of 70%. NaOCl is not applicable, and the applicability of the peroxide/detergent mixture needs further studies. First results indicate that the ranking of cleaning efficiency assessed in the laboratory test and in CIP of membrane elements is similar. Besides chemical ranking, the test can be used to optimize cleaning procedures related to duration, temperature, concentration and sequence of chemicals and mechanical shear. Additional (quantitative) knowledge on the relation of attached biomass with operational conditions problems of full-scale spiral-wound membrane processes and the efficacy of current cleaning practices is required to specify further cleaning research.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2012.09.013.

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